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Commercial Simian Virus Antisera That Inhibit Virus Replication in Primary Monkey Kidney Cell Cultures

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The incorporation of hyperimmune serum into cell culture medium to control endogenous viral infections of primary cells can have a significant effect on the replication of other viruses. When commercial simian virus 5 or simian virus 40 antiserum was used with primary monkey kidney cell cultures, we found a significant inhibition (>90%) of the replication of parainfluenza virus types 2 and 3 and reovirus type 1. In the viral diagnostic laboratory, the use of hyperimmune serum with primary monkey kidney cells may result in failure to isolate certain viruses if these cells are not first washed free of hyperimmune serum.

Simian virus 40 (SV40) is endogenous to several species of Asian macaques and was first discovered in cell cultures prepared from rhesus monkey kidneys (17). With the widespread use of primary monkey kidney cell cultures, it has become common practice to incorporate antiserum into culture medium to help control simian viruses which often make cell cultures unsuitable for viral isolations. For example, it has been recommended that 0.2 to 1.0% hyperimmune simian virus serum be used to control certain simian virus infections (3–5), and now it is common for commercial biological suppliers to add simian virus antiserum to primary monkey kidney cells. The incorporation of hyperimmune serum into cell culture medium could affect the isolation and growth of other viruses. The purpose of this report is to demonstrate that certain commercial lots of SV5 and SV40 antisera incorporated into cell culture medium have an inhibitory effect on the replication of parainfluenza virus types 2 and 3, reovirus type 1, and the simian rotavirus, SA11.

MATERIALS AND METHODS

Viruses and cell cultures. The viruses used in this study were reovirus type 1, poliovirus type 2, SA11 virus, SV40, and parainfluenza virus types 2 and 3. SA11 virus was obtained from H. H. Malherbe, The University of Texas Health Sciences Center at San Antonio. SV40 was obtained from the National Institute of Allergy and Infectious Diseases (NIAID) (Research Resources Branch), Bethesda, Md., and reovirus type 1 was obtained from NIAID and the American Type Culture Collection, Rockville, Md., whereas all other viruses were from the viral stocks of this laboratory. The cell cultures used were the fetal rhesus monkey kidney cell line MA104, obtained from H. H. Malherbe, the African green monkey kidney cell line BGM-70 (1), HeLa cells, and primary monkey kidney cells. All primary cells were purchased from M. A. Bioproducts, with and without SV5 or SV40 antiserum, and were used as obtained unless otherwise noted.

Viral quantitation and neutralization tests. For infectivity titrations, serial 10-fold dilutions of each viral preparation were inoculated into 2 to 10 tube cultures of the appropriate host cell culture system. Parainfluenza virus types 2 and 3 were detected by hemadsorption with guinea pig erythrocytes (0.25 to 0.50% suspensions). All other viruses were detected by monitoring for cytopathic effect. Fifty percent endpoints were determined by the method of Reed and Muench (13). Serum neutralization tests were performed by using methods previously described (6).

Hyperimmune sera. Three different lots of SV40 antiserum, obtained from M. A. Bioproducts, were tested for heterologous virus-inhibitory activity. Two lots were produced in goats, and one was produced in rabbits. Three different lots of SV5 antiserum were also tested for heterologous virus-inhibitory activity. A lot produced in horses was obtained from Flow Laboratories, and two lots (one rabbit and one goat) were purchased from M. A. Bioproducts. Each hyperimmune serum was added to cell cultures before viral titrations to give a final concentration of 0.5 to 1.0%. Primary monkey kidney cell cultures obtained directly from a commercial supplier and containing hyperimmune serum were also tested for virus-inhibitory activity.

RESULTS

Three different lots of commercial SV5 antiserum were tested for inhibition of parainfluenza virus type 3 replication. The lots (horse and rabbit) had no effect on the replication of parainfluenza virus type 3, whereas sera produced in goats significantly inhibited the replication of

parainfluenza virus type 3 in HeLa cells (Table 1). When the SV5 goat antiserum was tested for parainfluenza virus type 3 neutralizing antibody, the antiserum had a titer of 120 against 30 50% tissue culture infective doses of virus. Primary monkey kidney cells with SV5 antiserum also inhibited the replication of parainfluenza type 3. In fact, there was still significant inhibition of parainfluenza virus type 3 replication in primary monkey kidney cultures containing SV5 antiserum that had been incubated for 6 days at 37°C before being used (data not shown). Primary cell cultures refed with medium free of SV5 antiserum did not inhibit parainfluenza virus type 3 replication. The same SV5 goat antiserum that inhibited parainfluenza virus type 3 also significantly inhibited the replication of parainfluenza virus type 2 in HeLa cells (Table 1). Primary cell cultures obtained with SV5 goat antiserum also inhibited parainfluenza virus type 2 replication.

Three different commercial lots of SV40 antiserum were tested for virus-inhibitory activity against reovirus type 1. Two different lots of SV40 goat antiserum inhibited the replication of reovirus type 1 (Table 1). Goat SV40 antiserum did not inhibit the replication of poliovirus type 2 or SA11 virus, but it did inhibit the homologous SV40. Antiserum prepared in rabbits not only inhibited reovirus type 1, but also inhibited the replication of SA11 virus. The reovirus-inhibitory activity could not be removed by adsorption of an antiserum with primary monkey kidney cells (Table 1).

DISCUSSION

Three different commercial lots of SV5 antiserum were tested for virus-inhibitory activity, and one lot significantly inhibited (>90%) the replication of parainfluenza virus types 2 and 3. Primary monkey kidney cells, obtained directly from a biological supply house and having SV5 antiserum in the medium, also inhibited the replication of parainfluenza virus types 2 and 3. However, when the SV5 antiserum was removed by refeeding, no inhibitory activity was detected. Since hyperimmune simian virus serum may have antibody to heterologous viruses, primary cultures with hyperimmune serum should be washed before attempting parainfluenza virus isolations.

Goat and rabbit SV40 antisera used to control

TABLE 1. *Heterologous viral inhibition by commercial SV5 and SV40 antisera*

Antiserum[a]	Antiserum source	Virus (source)	Host cell	Titer/ml (50% tissue culture infective doses)	
				Antiserum[b]	No antiserum
SV5	Horse	Parainfluenza virus 3	HeLa	5.0, 5.0	5.0, 4.5
	Rabbit	Parainfluenza virus 3	HeLa	6.8, 6.8	7.0, 6.5
	Goat	Parainfluenza virus 3	HeLa	*<3.5, <2.5*	6.5, 6.2
	Goat	Parainfluenza virus 3	African green[c]	5.2, 3.5	6.8, 6.5
	Goat[d]	Parainfluenza virus 3	African green[c]	6.6, 6.2	6.5, 6.8
	Goat	Parainfluenza virus 2	HeLa	*2.0, <2.0*	5.8, 5.2
	Goat	Parainfluenza virus 2	African green[c]	*3.5*	5.5
SV40	Goat 1	Reovirus 1 (NIAID)	Rhesus[c]	*<0.5, 0.5*	3.2, 3.7
	Goat 1[e]	Reovirus 1	Rhesus[c]	*<0.5, <0.5*	2.2, 3.7
	Goat 1	Poliovirus 2	Rhesus[c]	2.5	2.0
	Goat 1	SA11 virus	MA 104	4.7	4.2
	Goat 1	SV40	African green[c]	*0.5, 2.7*	5.7, 6.2
	Goat 2	Reovirus 1 (NIAID)	Rhesus[c]	*<0.5*	3.2
	Goat 2	Reovirus 1 (ATCC)	BGM-70	7.6, 7.0	8.8, 8.5
	Goat 2	SA11 virus	MA104	4.2	4.2
	Rabbit	Reovirus 1	Rhesus[c]	*<0.5*	3.2
	Rabbit	SA11 virus	MA104	*3.2, 3.0*	4.2, 4.2

[a] A 1:100 to 1:200 dilution of either SV5 or SV40 antiserum was used with the continuous cells, whereas primary monkey kidney cells were used as received, with or without antiserum.

[b] A titer in italics represents a significant inhibition of viral replication in the presence of heterologous antiserum when compared with viral replication without antiserum.

[c] Primary monkey kidney cells.

[d] Goat SV5 antiserum was removed from the cultures by refeeding them with maintenance medium containing 5% fetal bovine serum.

[e] Antiserum was adsorbed overnight at 4°C with packed primary rhesus monkey kidney cells.

latent SV40 infections in primary monkey kidney cells inhibited reovirus type 1 replication. This was not surprising, since reoviruses have been isolated from a variety of species, including humans, mice, dogs, cats, sheep, cattle, swine, horses, monkeys, and chimpanzees (12). The reovirus-inhibitory activity probably was not due to anticellular antibody, because activity was not removed by adsorption with primary monkey kidney cells. Cultures used for reovirus isolation also should be washed free of serum (12, 14). This is especially important because some suppliers are using calf serum with cell cultures.

SA11 virus was inhibited by an SV40 rabbit antiserum probably because of inhibitors or antibodies for SA11 or a closely related rotavirus. Rotaviruses have been isolated from humans, calves, mice, piglets, foals, lambs, rabbits, deer, antelopes, monkeys, apes, bison turkeys, chickens, goats, and kittens (7). It is possible that rabbits can be infected with SA11 virus or a closely related rotavirus. However, Petric et al. (11) found that rabbits inoculated with SA11 virus or a human rotavirus were not infected; they suggested that perhaps the rabbits were too old for infections to occur. It has been reported that a human rotavirus can infect piglets, lambs, and monkeys (7) and that human sera contain high levels of neutralizing antibody to the calf rotavirus (8). Also, hyperimmune serum for a human rotavirus had a low level of cross-neutralizing activity for a calf rotavirus (9). Thus, various sera could inhibit rotavirus replication, and a serum-free medium should be used for the isolation of these viruses.

We feel that there are no satisfactory methods for preventing or completely controlling endogenous viral infections of primary cells. Once a latent viral infection has been detected in a cell culture, that culture should not be used for viral isolations. If hyperimmune serum is used in efforts to control latent simian virus infections in primary monkey kidney cells, the cultures should be washed or at least refed with serum-free medium (2, 10, 12, 14). It is our experience that not all viral diagnostic laboratories follow this precaution of using serum-free medium for certain viral isolations, and some workers do not recommend using serum-free medium (5). It should be emphasized that the use of simian virus antiserum in cell culture media may not even be a reliable method for suppressing endogenous viruses (15); its inclusion may only give a false sense of security (16) and also might inhibit heterologous viruses. Orthomyxovirus, paramyxovirus, and reovirus isolations in cell culture are enhanced by the use of serum-free cell culture medium. Thus, for isolating certain viruses, serum-free medium should be used or the serum should at least be screened to ensure that it will not inhibit the recovery of viruses from clinical specimens.

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